

IN THE CLAIMS

The following listing of claims will replace all prior versions, and listings, of claims in this application.

Listing of the Claims:

Claims 1-21 (Cancelled).

22. (Original) An isolated polypeptide comprising SEQ ID NO:2.

Claims 23-25 (Cancelled).

26. (Currently Amended) An N-acetylglucosamine-1-phosphotransferase (GlcNAc-phosphotransferase) comprising an  $\alpha$  subunit, a  $\beta$  subunit and a site-specific proteolytic cleavage site interposed between said  $\alpha$  and  $\beta$  subunits, wherein said site-specific proteolytic cleavage site is not endogenous to GlcNAc-phosphotransferase,

wherein said  $\alpha$  subunit is encoded by nucleotides 165 to 2948 of SEQ ID NO:3, or a sequence that hybridizes under stringent conditions to the complement of nucleotides 165 to 2948 of SEQ ID NO:3, wherein said stringent conditions comprise hybridization in 50% formamide, 1M NaCl, 1% SDS at 37°C and washing in 0.1 X SSC at 60 to 65°C and which encodes a protein when combined with a  $\beta$  subunit protein encoded by nucleotides 2949 to 2932 of SEQ ID NO:3 has GlcNAc-phosphotransferase activity; and

wherein said  $\beta$ -subunit is encoded by nucleotides 2949 to 3932 of SEQ ID NO:3, or a sequence that hybridizes under stringent conditions to the complement of nucleotides 2949 to 3932 of SEQ ID NO:3, wherein said stringent conditions comprise hybridization in 50% formamide, 1M NaCl, 1% SDS at 37°C and washing in 0.1 X SSC at 60 to 65°C and which encodes a protein when combined with an  $\alpha$  subunit protein encoded by nucleotides 165 to 2948 of SEQ ID NO:3 has GlcNAc-phosphotransferase activity.

Claims 27-29 (Cancelled).

30. (Previously Presented) The GlcNAc-phosphotransferase of Claim 26, wherein said  $\alpha$ -subunit comprises amino acids 1-928 of SEQ ID NO:4.

31. (Previously Presented) The GlcNAc-phosphotransferase of Claim 26, wherein said  $\beta$  subunit comprises amino acids 1 to 328 of SEQ ID NO:5.

32. (Original) The GlcNAc-phosphotransferase of Claim 26, wherein said GlcNAc-phosphotransferase further comprises a  $\gamma$  subunit.

Claim 33 (Cancelled).

34. (Original) The GlcNAc-phosphotransferase of Claim 32, wherein said  $\gamma$  subunit comprises the amino acid sequence of SEQ ID NO:7.

35. (Currently Amended) The GlcNAc-phosphotransferase of Claim 26, wherein said site-specific proteolytic cleavage site is selected from the group consisting of a Furin proteolytic cleavage site, a Factor Xa proteolytic cleavage site, a Enterokinase proteolytic cleavage site, and a ~~Genenase I~~ histidine-tyrosine proteolytic cleavage site.

36. (Original) The GlcNAc-phosphotransferase of Claim 35, wherein said site-specific proteolytic cleavage site is a Furin proteolytic cleavage site.

37. (Previously Presented) The GlcNAc-phosphotransferase of Claim 36, wherein said Furin proteolytic cleavage site comprises SEQ ID NO:24.

Claims 38-55 (Cancelled).

56. (Previously Presented) A method of phosphorylating a lysosomal hydrolase protein comprising an asparagine-linked oligosaccharide with a high mannose structure, the method comprising contacting said lysosomal hydrolase protein with the isolated polypeptide of Claim 22 for a time and under conditions suitable to produce a phosphorylated lysosomal hydrolase protein.

Claims 57-69 (Cancelled).

70. (Currently Amended) The method of Claim 56, wherein said lysosomal hydrolase protein is selected from the group consisting of  $\alpha$ -glucosidase,  $\alpha$ -L-iduronidase,  $\alpha$ -galactosidase A, arylsulfatase, N-acetylgalactosamine- $\alpha$ -sulfatase,  $\beta$ -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase,  $\beta$ -glucuronidase, Heparan N-sulfatase, N-Acetyl- $\alpha$ -glucosaminidase, Acetyl CoA- $\alpha$ -glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside sialidase, Acid  $\beta$ -galactosidase GM<sub>1</sub>-Ganglioside, Acid  $\beta$ -galactosidase, Hexosaminidase A, Hexosaminidase B,  $\alpha$ -fucosidase,  $\alpha$ -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase, Sphingomyelinase, and Glucocerebrosidase  $\beta$ -Glucosidase.

71. (Previously Presented) The method of Claim 56, further comprising contacting said phosphorylated protein with an isolated N-acetylglucosamine-1-phosphodiester-N-Acetylglucosaminidase (phosphodiester  $\alpha$ -GlcNAcase).

72. (Previously Presented) The method of Claim 71, wherein said phosphodiester  $\alpha$ -GlcNAcase comprises the amino acid sequence of SEQ ID NO:18.

73. (Currently Amended) The method of Claim 71, wherein said phosphodiester  $\alpha$ -GlcNAcase is encoded by a nucleotide sequence comprising SEQ ID NO:17 or a nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:17, wherein said stringent conditions comprise hybridization in 50% formamide, 1M NaCl, 1% SDS at 37°C and washing in 0.1 X SSC at 60 to 65°C, and which encodes a protein with phosphodiester  $\alpha$ -GlcNAcase activity.

74. (Previously Presented) The method of Claim 56, wherein prior to said contacting the method comprises: culturing a host cell which comprises a polynucleotide encoding the

polypeptide for a time under conditions suitable for expression of the polypeptide; and isolating said polypeptide.

75. (Cancelled)

76. (Currently Amended) A method of phosphorylating a lysosomal hydrolase protein comprising an asparagine-linked oligosaccharide with a high mannose structure, the method comprising contacting said protein with the GlcNAc phosphotransferase of Claim 26 for a time and under conditions suitable to produce a phosphorylated protein.

Claims 77-80 (Cancelled).

81. (Previously Presented) The method of Claim 76/78, wherein said  $\alpha$ -subunit comprises amino acids 1-928 of SEQ ID NO:4.

82. (Previously Presented) The method of Claim 76/78, wherein said  $\beta$  subunit comprises amino acids 1 to 328 of SEQ ID NO:5.

83. (Previously Presented) The method of Claim 76/78, wherein said soluble GlcNAc-phosphotransferase further comprises a  $\gamma$  subunit.

84. (Currently Amended) The method of Claim 83, wherein said  $\gamma$  subunit is encoded by SEQ ID NO:6, or a nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:6, wherein said stringent conditions comprise hybridization in 50% formamide, 1M NaCl, 1% SDS at 37°C and washing in 0.1 X SSC at 60 to 65°C.

85. (Previously Presented) The method of Claim 83, wherein said  $\gamma$  subunit comprises the amino acid sequence of SEQ ID NO:7.

86. (Currently Amended) The method of Claim 76, wherein said site-specific proteolytic cleavage site is selected from the group consisting of a Furin proteolytic cleavage

site, a Factor Xa proteolytic cleavage site, a Enterokinase proteolytic cleavage site, and a Genenase I-histidine-tyrosine proteolytic cleavage site.

87. (Previously Presented) The method of Claim 86, wherein said site-specific proteolytic cleavage site is a Furin proteolytic cleavage site.

88. (Previously Presented) The method of Claim 87, wherein said Furin proteolytic cleavage site comprises SEQ ID NO:24.

Claim 89 (Cancelled).

90. (Currently Amended) The method of Claim 76 ~~89~~, wherein said lysosomal hydrolase protein is selected from the group consisting of  $\alpha$ -glucosidase,  $\alpha$ -L-iduronidase,  $\alpha$ -galactosidase A, arylsulfatase, N-acetylgalactosamine- $\alpha$ -sulfatase,  $\beta$ -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase,  $\beta$ -glucuronidase, Heparan N-sulfatase, N-Acetyl- $\alpha$ -glucosaminidase, ~~Acetyl CoA- $\alpha$ -glucosaminide N-acetyl transferase, N-acetyl-~~ glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, ~~Arylsulfatase A-Cerebroside~~, Ganglioside sialidase, Acid  $\beta$ -galactosidase  $G_{M_1}$ -Ganglioside, Acid  $\beta$ -galactosidase, Hexosaminidase A, Hexosaminidase B,  $\alpha$ -fucosidase,  $\alpha$ -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase, Sphingomyelinase, and Glucocerebrosidase  $\beta$ -Glucosidase.

91. (Previously Presented) The method of Claim 76, further comprising contacting said phosphorylated protein with an isolated phosphodiester  $\alpha$ -GlcNAcase.

92. (Previously Presented) The method of Claim 91, wherein said phosphodiester  $\alpha$ -GlcNAcase comprises the amino acid sequence of SEQ ID NO:18.

93. (Currently Amended) The method of Claim 91, wherein said phosphodiester  $\alpha$ -GlcNAcase is encoded by a nucleotide sequence comprising SEQ ID NO:17 or a nucleotide

sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:17, wherein said stringent conditions comprise hybridization in 50% formamide, 1M NaCl, 1% SDS at 37°C and washing in 0.1 X SSC at 60 to 65°C.

94. (Previously Presented) The method of Claim 76, wherein prior to said contacting the method comprises: culturing a host cell which comprises a polynucleotide encoding the GlcNAc-phosphotransferase for a time under conditions suitable for expression of the GlcNAc-phosphotransferase; and isolating said GlcNAc-phosphotransferase.

95. (Previously Presented) The method of Claim 76, wherein prior to said contacting the method comprises culturing a host cell which comprises a polynucleotide encoding the GlcNAc-phosphotransferase for a time under conditions suitable for expression of the GlcNAc-phosphotransferase; isolating said GlcNAc-phosphotransferase; cleaving said isolated GlcNAc-phosphotransferase with a proteolytic enzyme specific for said proteolytic cleavage site; and mixing said  $\alpha$  and  $\beta$  subunits with a  $\gamma$  subunit of GlcNAc-phosphotransferase.